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On the Giant Octopus (*Octopus giganteus*) and the Bermuda Blob: Homage to A. E. Verrill

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"The substance looks like blubber, and smells like blubber and it is blubber, nothing more or less."
—F. A. Lucas, 1897

Abstract. We have obtained samples of two large carcasses. One washed up on a beach in St. Augustine, Florida, in 1896 and has been occasionally attributed to a species of gigantic octopus (*Octopus giganteus*). The other carcass washed up on Bermuda in 1988 and has remained unidentified, although its gross morphology, except for a much smaller total mass, was remarkably similar to the Florida carcass. We have subjected both samples to electron microscopic and biochemical analyses. Our results show that both carcasses are masses of virtually pure collagen. Furthermore, neither sample has the biochemical characteristics of invertebrate collagen, nor the collagen fiber arrangement of octopus mantle. Instead, they are large pieces of vertebrate skin, the Bermuda sample from a poikilotherm and the Florida sample from a huge homiotherm. We conclude that there is no evidence to support the existence of *Octopus giganteus*.

Introduction

The first evidence that seemed to document the existence of a species of gigantic octopus washed ashore on an oceanic beach at St. Augustine, Florida, late in the year of 1896. The so-called carcass was taken in charge by a local physician, Dr. DeWitt Webb, who was also president of the St. Augustine Scientific Society (Wood, 1971). Webb set about to photograph the body, dig it out of the sand, haul it up above the high tides (a task that required the efforts of several horses and men), and to inform the scientific community of its existence. Because Webb believed the carcass to be the remains of a huge octopus, one of the scientists he contacted was the preeminent invertebrate naturalist, Professor A. E. Verrill at Yale (Fig. 1). Based at first only upon a letter from Webb, Verrill reported the finding, speculating that it might be a specimen of *Architeuthis* (Verrill, 1897a). Shortly thereafter, having received additional correspondence and photographs from Webb, Verrill concluded that it was a "true *Octopus*, of colossal size . . . one of those upon which the sperm whale feeds regularly." On the basis of the descriptive and photographic evidence, Verrill proposed to name the species *Octopus giganteus* (Verrill, 1897b). However, almost immediately, Verrill changed his mind. Based upon more photographs, measurements, and descriptions of the carcass after it had been entirely unearthed from the beach sand, along with several formalin-preserved pieces of the tissue, Verrill retracted his rapidly drawn, initial conclusions, writing that "the creature could not have been an *Octopus*" (Verrill, 1897c, d). Instead, Verrill, together with some other biologists of the time, came to the conclusion that the carcass was from a large vertebrate, most likely a whale (Lucas, 1897; Verrill, 1897d, e). However, other biologists, notably Dr. William H. Dall, then the Curator of Mollusks at the National Museum of Natural History, still favored Verrill's original species diagnosis according to correspondence between him, Webb, and Verrill (archived at the Smithsonian Institution, Washington, DC).

Although Verrill disavowed his species description of *Octopus giganteus* several times, the carcass was never properly identified. The matter rested quietly for 70 years,

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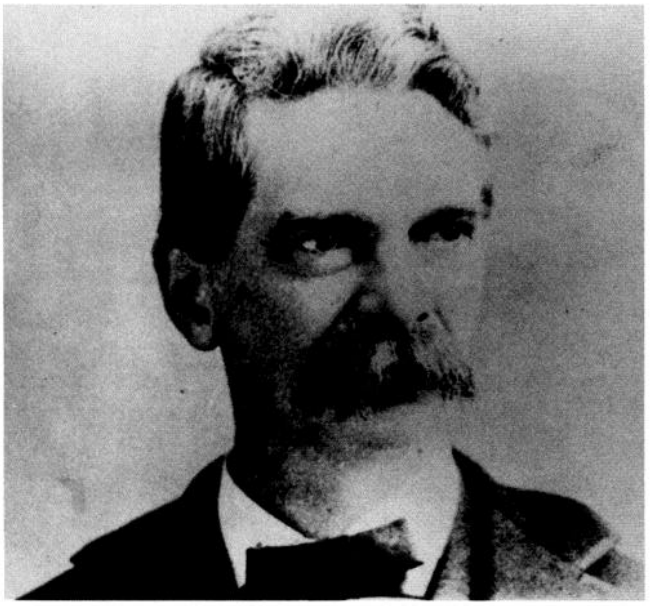

Figure 1. A. E. Verrill. Courtesy of the Marine Biological Laboratory Archives.

and then a report appeared stating "it can be safely said that the gigantic mass of tissue that washed up on the beach at St. Augustine in 1896 was the remains of an octopus . . . 200 feet . . ." between tentacle tips (Wood, 1971). That report continued to detail the chronology of the events summarized above and also indicated that sizable pieces of the carcass had been preserved and were held at the Smithsonian Institution. A cursory histological comparison of the tissue from the Smithsonian with that of "contemporary" squid and octopus was carried out. Although the specific squid and octopus tissues that were compared to the Florida carcass were not reported, no cellular structure was found in any of the tissues. Instead, a connective tissue fiber network in all three tissues was revealed with polarized light. The conclusions reached were that none of the samples looked mammalian, that the St. Augustine tissue looked much more like the octopus fiber network than that of the squid, and therefore, "the St. Augustine sea monster was in fact an octopus" (Gennaro, 1971). Because the report of these histological studies was written for a general, rather than scientific audience, it lacked a rigorous description of protocol and observations.

The matter rested again, this time for another decade and a half, until the appearance of a report about the amino acid composition of an acid hydrolysate of the St. Augustine tissue, by now almost 100 years old. Although neither hydroxylysine nor hydroxyproline concentrations were determined, the amino acid composition suggested that the St. Augustine tissue was likely to have been a huge mass of collagen (Mackal, 1986), explaining the lack of cellular structure found in it by Gennaro (1971) (but not the lack of such structure in the "contemporary" tissues) and the persistence of the carcass as it lay on the beach. (According to the correspondence between Webb and Dall, the carcass was still on the beach on March 17, 1897, almost 4 months after the initial discovery.) Mackal (1986) concluded that the amino acid data together with some inconclusive Cu and Fe measurements "support the original identification of the tissue and carcass by A. E. Verrill as an exceptionally large cephalopod, probably octopus, not referable to any known species," in spite of both Verrill's change of mind and the complete lack of a suitable test of taxonomic relationships in Mackal's data. In the end, while the existence of the St. Augustine carcass is well documented and the discovery often cited [most recently in the popular press (Ellis, 1994) and in a biological science text book (Milne, 1995)], there is no unequivocal evidence at all that it belonged to a giant octopus or, indeed, to any particular species.

During the summer of 1988 another carcass washed into a lagoon on the island of Bermuda. This unrecognizable carcass ($\sim$2.50 $\times$ 1.25 $\times$ 0.30 m), immediately labeled the "Bermuda Blob" by the popular press, was photographed and sampled by Teddy Tucker, a renowned local diver and fisherman who often works with scientists on Bermuda. While considerably smaller than the St. Augustine discovery, the Bermuda carcass fit Verrill's description exactly, on a gross level. "No bones or hard parts. . . . Instead of being muscular . . . [the tissues] are firm, tough and elastic, and composed mainly of much interlaced fibers and large bundles of tough fibrous, white connective tissue. [The tissue is] difficult to cut or tear apart . . . . Some large irregular canals permeate the [tissue]. These may have contained blood vessels originally. From the inner surface of some of the pieces large cords of elastic fibers proceeded inward" (Verrill, 1897c). Gennaro's additional description of the St. Augustine tissue also fit the Bermuda carcass exactly. "White as soap . . . the connective tissue was so tough that it dulled four blades . . . the same homogenous, tough, white, fibrous texture [throughout]" (Gennaro, 1971).

We have been able to obtain small pieces of both the St. Augustine and Bermuda carcasses. We have subjected both to electron microscopic examination as well as biochemical analyses to test the similarity between the two tissue masses and to determine their taxonomic origin. In addition, we have carried out light and electron microscopic examinations of octopus (*Bathypolypus arcticus*) mantle tissue and humpback whale (*Megaptera novaeangelae*) blubber.

Materials and Methods

Electron microscopy

Bermuda and St. Augustine Carcasses. Specimens from both the Bermuda and St. Augustine tissue masses were

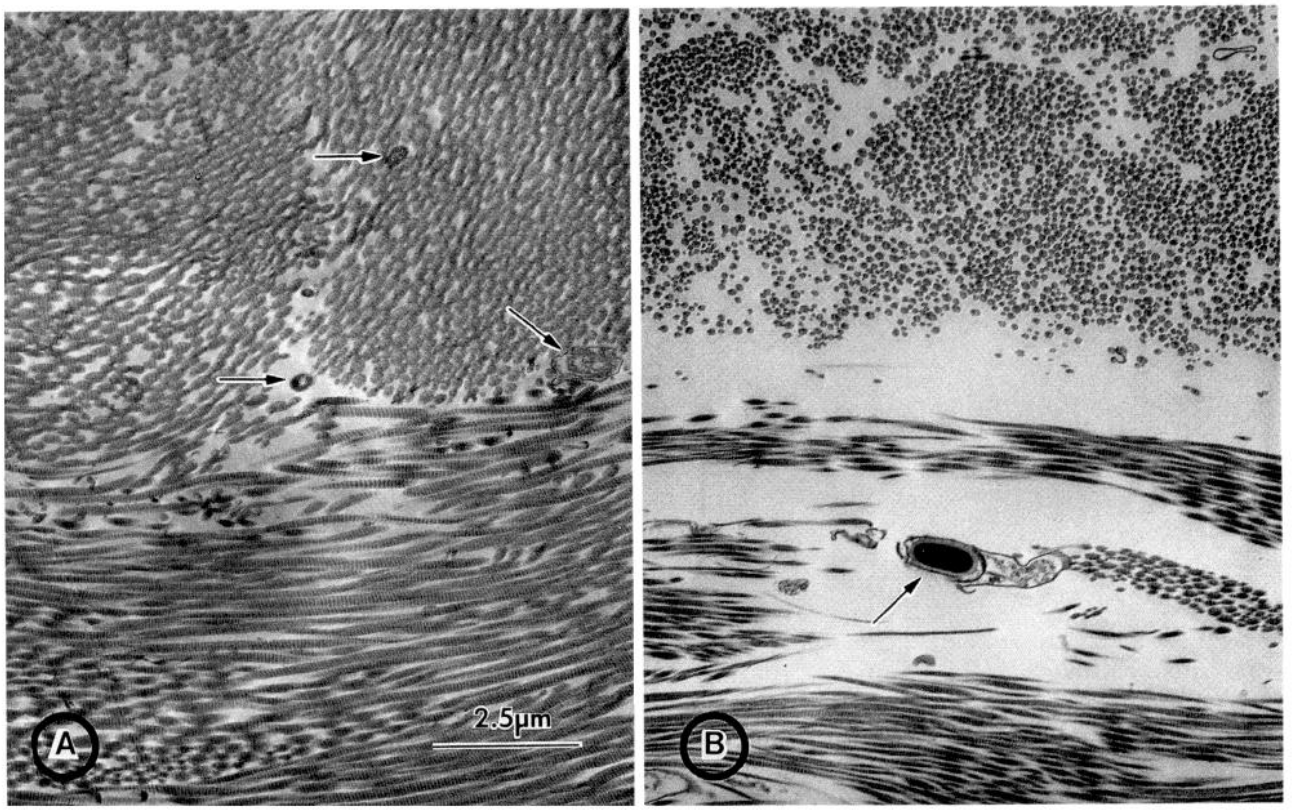

Figure 2. Low magnification transmission electron micrographs of sections of the Bermuda (A) and St. Augustine (B) carcasses. The collagen fibers of both tissues run in layers that are perpendicular to each other. Within each layer the fibers appear to be organized in bundles (see the upper half of A). This type of fiber organization is typical of skin collagen (see Discussion). Other than the fibers, no other cellular elements were found. Bacteria and bacterial spores (arrows) were scattered throughout the fiber layers in both samples.

prepared for electron microscopic examination. Although the exact composition of the original preservation medium was unknown for either tissue, the distinct odor of formalin was obvious in both. Indeed, Webb, in his correspondence with Dall, indicated that he had put several pieces of the body in formalin before sending them off to both Dall and Verrill (cited in Wood, 1971). We cut several pieces (1 mm^3) from each of the original tissue samples and placed them directly into 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 *M* cacodylate buffer containing 0.3 *M* sucrose (~1100 mosm) (Bermuda sample) or 2% glutaraldehyde in 0.15 *M* cacodylate buffer containing 0.58 *M* sucrose (~1100 mosm) (Florida sample). The tissue samples remained in the fixative for several days. After fixation, the tissue pieces were post-fixed in 2% OsO_4 in the cacodylate-sucrose buffer, treated *en bloc* with 2% aqueous uranyl acetate, dehydrated in an ethanol series (35–100%), and embedded in epoxy resin (Spurr's medium). Thin sections showing a silver interference color were cut with a diamond knife on an ultramicrotome (Reichert, Ultracut E). The sections were mounted on copper grids and stained with 2% uranyl acetate for 5 min, followed by 0.2% lead citrate for 1.5 min (Venable and Coggeshall, 1965). The stained sections were examined with a transmission electron microscope (Zeiss EM 10 CA) at 80 kV.

Octopus mantle. The specimen of *B. arcticus* (USNM catalogue #884184) that provided a mantle tissue sample had been collected by trawl off the New Jersey coast during a 1981 cruise of the R/V *Delaware II* and had been placed immediately into formalin upon its capture. At some point, the octopus was transferred into isopropyl alcohol and had been stored in that solution until we were given access to it. We cut a section of the mantle off the octopus, rehydrated it and placed it into 2% glutaraldehyde. The tissue was then prepared for electron microscopy, as described above for the Florida sample. For light microscopy, thick sections were cut from the same specimen, stained with Richardson's stain (a mixture of methylene blue and Azure II), and viewed with bright-field optics (Zeiss, Photomicroscope II).

Whale blubber. We cut a small sample of blubber from a much larger piece that came from a male humpback whale that had died at sea and washed onto a beach in

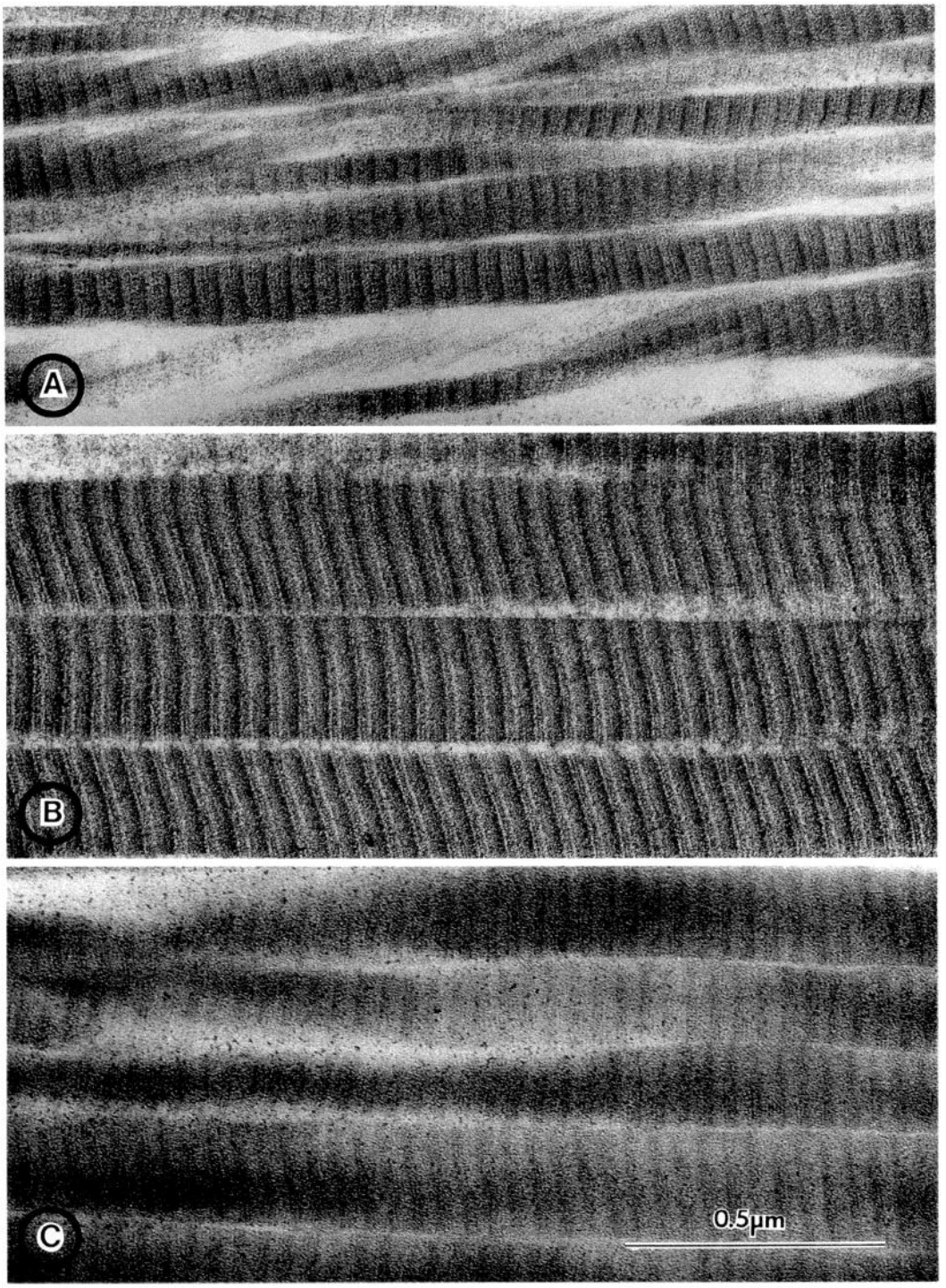

Figure 3. Higher magnification transmission electron micrographs showing the periodicity along the fibers of the St. Augustine collagen (A), rat tail tendon collagen (B), and the Bermuda collagen (C), all at the same magnification. The fibers of the St. Augustine and Bermuda samples are thinner than those in the rat tail tendon—a characteristic of skin collagen. The somewhat indistinct banding pattern of the Bermuda fibers is likely due to the poor original fixation. The dense deposits in this sample also derive from the original fixative solution.

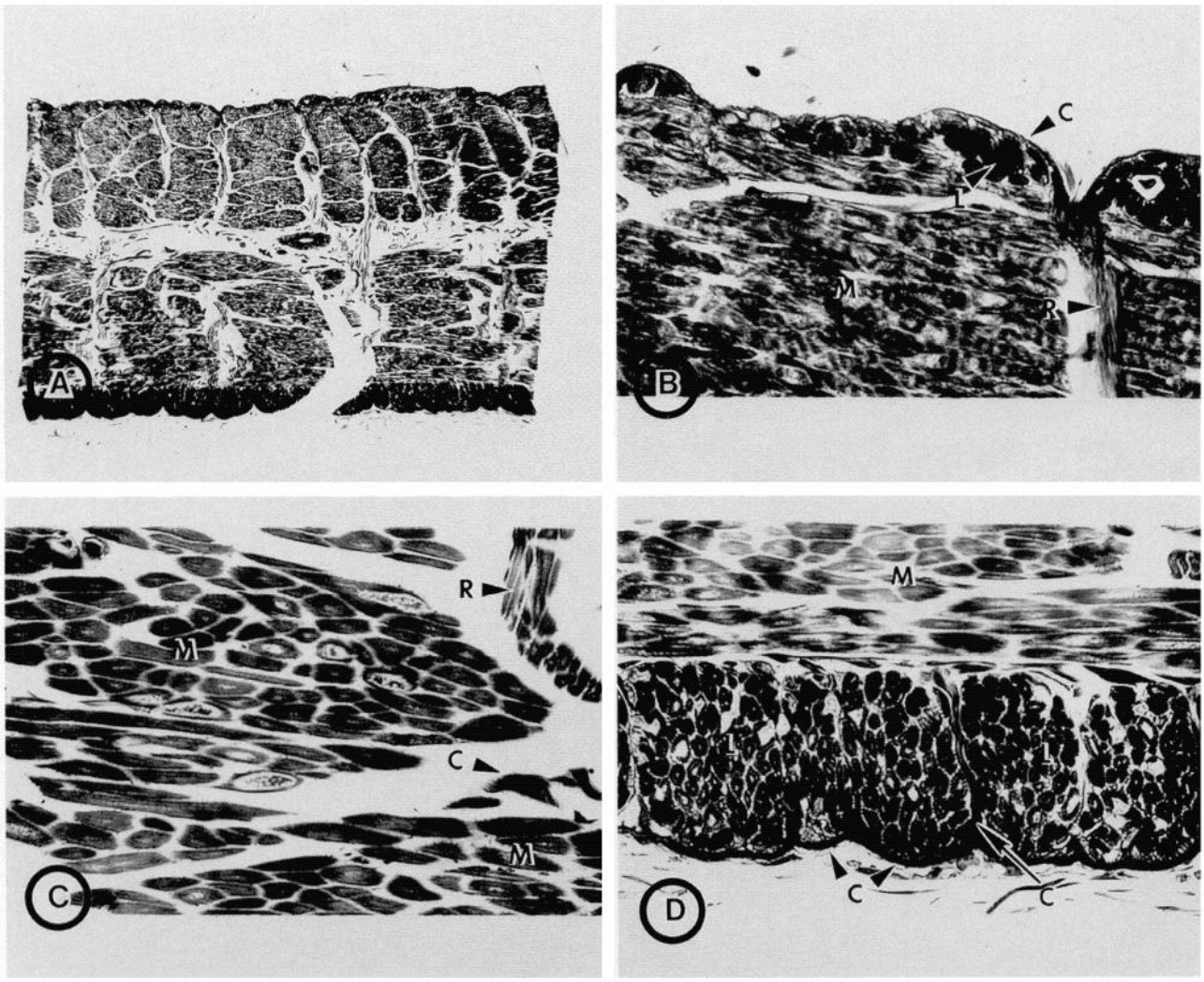

Figure 4. Light micrographs of cross sections taken through the width of the mantle of *Bathypolypus arcticus*. The epidermis, which consists of an epithelium underlain with a thin layer of dispersed collagen fibers, has been removed. (Micrograph A) The mantle consists of two primary layers of muscle bundles of about equal width. These layers are separated by a space that contains blood vessels (oval structure in the center) (magnification = 62×). (Micrograph B) The outer surface of the mantle consists of small bundles of longitudinal muscles (L) covered by a very thin layer of collagen (arrowhead C). Underlying the longitudinal muscles are muscle bundles that also run longitudinally, but containing individual fibers within each bundle that are not parallel to each other (M). Interspersed between these deeper bundles run thin radial muscles (R) which span the width of the mantle (see micrograph A, also) (magnification = 390×). (Micrograph C) Below the blood vessel layer run additional muscle bundles containing fibers with a wide array of orientations (M). Small bundles of collagen (arrowheads C) containing fibers that run parallel to each other occur occasionally between the muscle bundles. The hollow, tubular structure of the muscle cells is evident in this section (magnification = 390×). (Micrograph D) The inner surface of the mantle is also covered by a thin layer of collagen (arrowhead C) that extends upward between (arrow C) adjacent bundles of the longitudinal muscles (L). Although not shown in this section, the radial muscles insert between the longitudinal muscle bundles (see micrograph A). The longitudinal muscle bundles are much larger on this aspect of the mantle than those on the outer surface (compare with micrograph B). Immediately above the longitudinal muscles runs a thin bundle of circularly oriented muscle fibers (magnification = 390×).

Virginia Beach County, Virginia, in October 1992. According to the Virginia Museum of Science collection record, this carcass was 906 cm long (about 200 cm longer than the St. Augustine carcass) and only moderately decayed. The original piece of the blubber, still attached to the epidermis, had been preserved in formalin and deposited at the Smithsonian (catalogue #VMSM 921025). Our sample was transferred into 2% glutaraldehyde, small

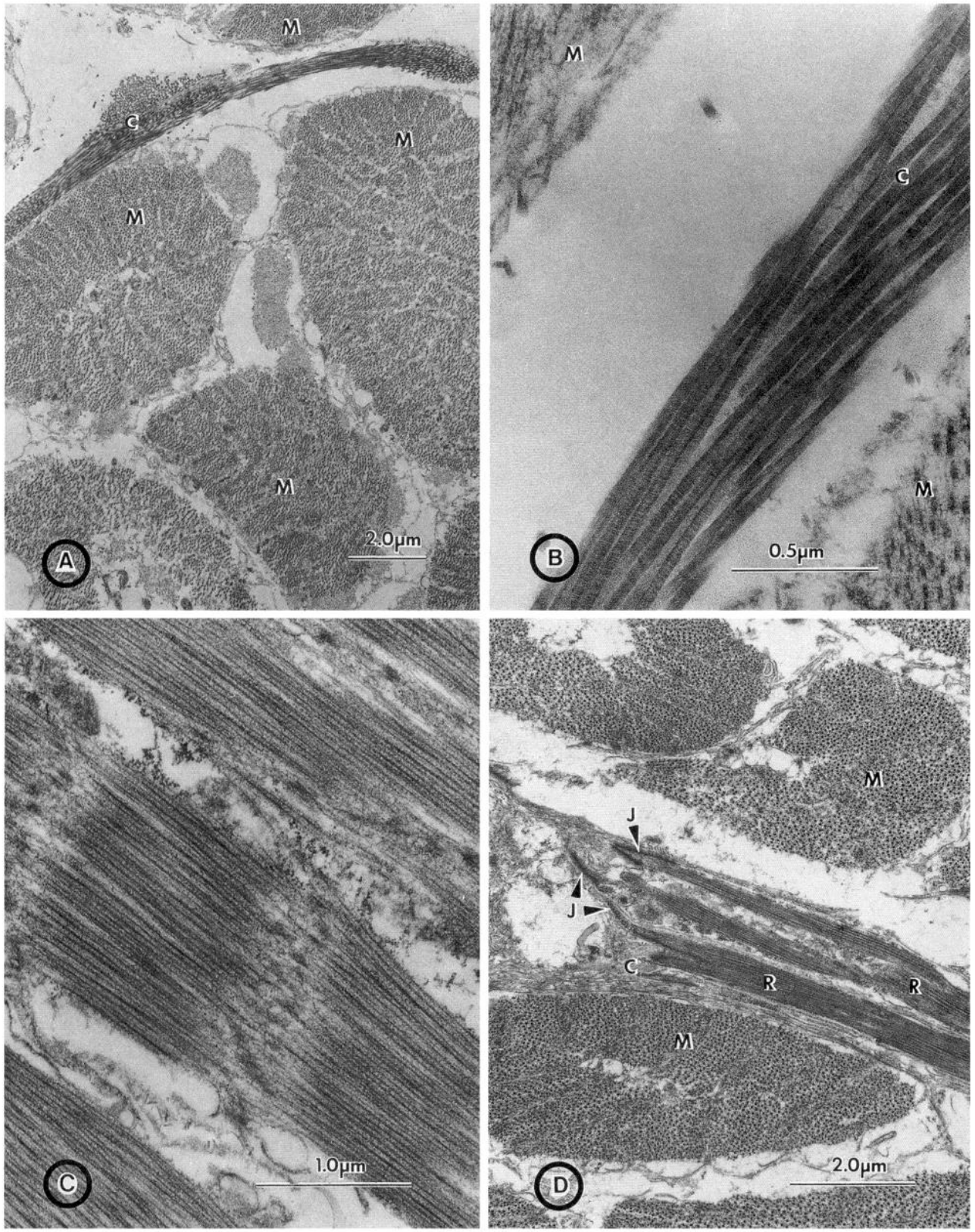

Figure 5. Electron micrographs of *Bathypolypus arcticus* mantle. (Micrograph A) This cross section shows the arrangement of the contractile proteins in bundles that radiate from the hollow center of each tubular muscle cell (M). Adjacent muscle fibers are separated by occasional bundles of collagen fibers (C). (Micrograph B) The fibers within the collagen bundles (C) always run parallel to each other. Adjacent layers of perpendicularly running collagen fibers were never seen. (Micrograph C) The contractile proteins within

pieces were cut from immediately under the epidermis, the middle, and the inner regions of the blubber, and all were prepared for electron microscopy as described above for the Florida sample.

Rat tail tendon and skin. Because our initial microscopic examination of sections from the carcasses found fibers that resembled collagen, we proceeded to measure the periodicity of the banding pattern of the fibers to confirm that identification. We used collagen fibers from rat tail tendon as an internal standard for these measurements. A piece of tail was obtained from a white rat that had been decapitated and immediately frozen for other experimental purposes. The tail was thawed, skinned, and the tail tendon removed. Pieces (1 mm^3) were cut from both the skin and tendon and fixed in 2% glutaraldehyde in 0.12 *M* phosphate buffer (pH 7.4). Following initial fixation, the tissue pieces were washed in the above buffer and then postfixed in 2% OsO_4 in the phosphate buffer. Subsequent preparative steps were the same as described above for the Florida sample.

Amino acid analysis

In the case of both samples, the small piece of tissue that remained following the microscopy (256 mg, Bermuda, 216 mg, St. Augustine) was soaked in several changes of artificial seawater (940 mosm) overnight at 4°C to wash out the preservative solution. The tissue was then cut into pieces (2 mm^3) and hydrolyzed for 24 h in 6 *N* HCl at 100°C. Both samples dissolved within 15 min of being placed into the acid. The hydrolysate was neutralized with NaOH and the amino acids extracted with an equal volume of 95% ethanol. The extract was centrifuged at 20,000 × *g* (4°C), and the supernatant was freeze-dried overnight. The residue was dissolved in 0.2 *N* lithium citrate buffer (pH 2.2), and the amino acid composition of this last solution determined with an automatic amino acid analyzer with ninhydrin detection (Beckman, System Gold). Amino acid concentrations were calculated by the System Gold software with norleucine as an internal standard, and then converted to residues/1000 residues for each individual amino acid. These data were graphically compared to the amino acid compositions of the collagens of 97 species from diverse phyla, according to the protocol described by Matsumura (1972).

Briefly, the Matsumura protocol consists of calculating the sum of each of the hydrophobic (valine, methionine, leucine, isoleucine, tyrosine, and phenylalanine), hydroxylated (hydroxyproline, threonine, serine, tyrosine, and hydroxylysine), and polar (aspartate, glutamate, hydroxylysine, lysine, histidine, ornithine, and arginine) amino acid residues per 1000 in the collagen hydrolysate. Each sum was divided by the grand sum of the three amino acid groups and then multiplied by 1000 to yield a set of three of Matsumura's *R* values. A vector **R** is then plotted on a triangular coordinate graph using the *R* values ($R_{\text{hydrophobic}}$, $R_{\text{hydroxylated}}$, and R_{polar}) as coordinates (Matsumura, 1972).

Results

The electron microscopy and the amino acid analysis indicate that both the Bermuda and St. Augustine carcasses are made up almost exclusively of collagen fibers.

The pieces of tissue from the Bermuda and St. Augustine carcasses contain layers of fibers showing banding patterns that are characteristic of collagen, a few scattered bacteria and bacterial spores, and no other cellular structures (Fig. 2). In both specimens, adjacent layers of the collagen fibers run perpendicular to each other. Although the fixation of the Bermuda sample is not very good, longitudinal sections of the two specimens appear very similar at low, and even at high, magnifications. Certainly, the banding periodicity along the fibers is similar both to each other and to rat tail tendon collagen (Fig. 3). We measured the distance between the major periods along several fibers in several sections at both low and high magnification. The averages were 54.3 nm (±2.72 SD, n = 150 measurements) for the St. Augustine collagen and 57.9 nm (±5.37 SD, n = 154 measurements) for the Bermuda fibers. Although these values are slightly less than the usually published periodicity for collagen banding (60 nm), control samples of both rat tail tendon (Fig. 3B) and rat skin collagen yielded a banding periodicity of 55.7 nm (±5.6 SD) with our protocol. The diameter of the individual fibers was also determined from micrographs of both cross sections and longitudinal sections. The average for the St. Augustine collagen was 109 nm (±25.2 SD, n = 68), 156 nm (±34.7 SD, n = 106) for the Bermuda collagen, and 173 nm (±82.0 SD, n = 31) for rat tail tendon collagen.

Microscopic examination of *Bathypolypus arcticus* mantle revealed a structure that is dramatically different from that of the two carcasses. In particular, the massive, perpendicular collagen fiber arrangement characteristic of the two carcasses is completely absent in the octopus

the sarcomeres of the mantle muscle cells are not in register. (Micrograph D). The radial muscles (R) that span the width of the mantle between the rest of the mantle musculature (M) are attached to the inner and outer layer of collagen (C) by junctional complexes (J). The dorsal surface of the mantle lies to the left of this micrograph.

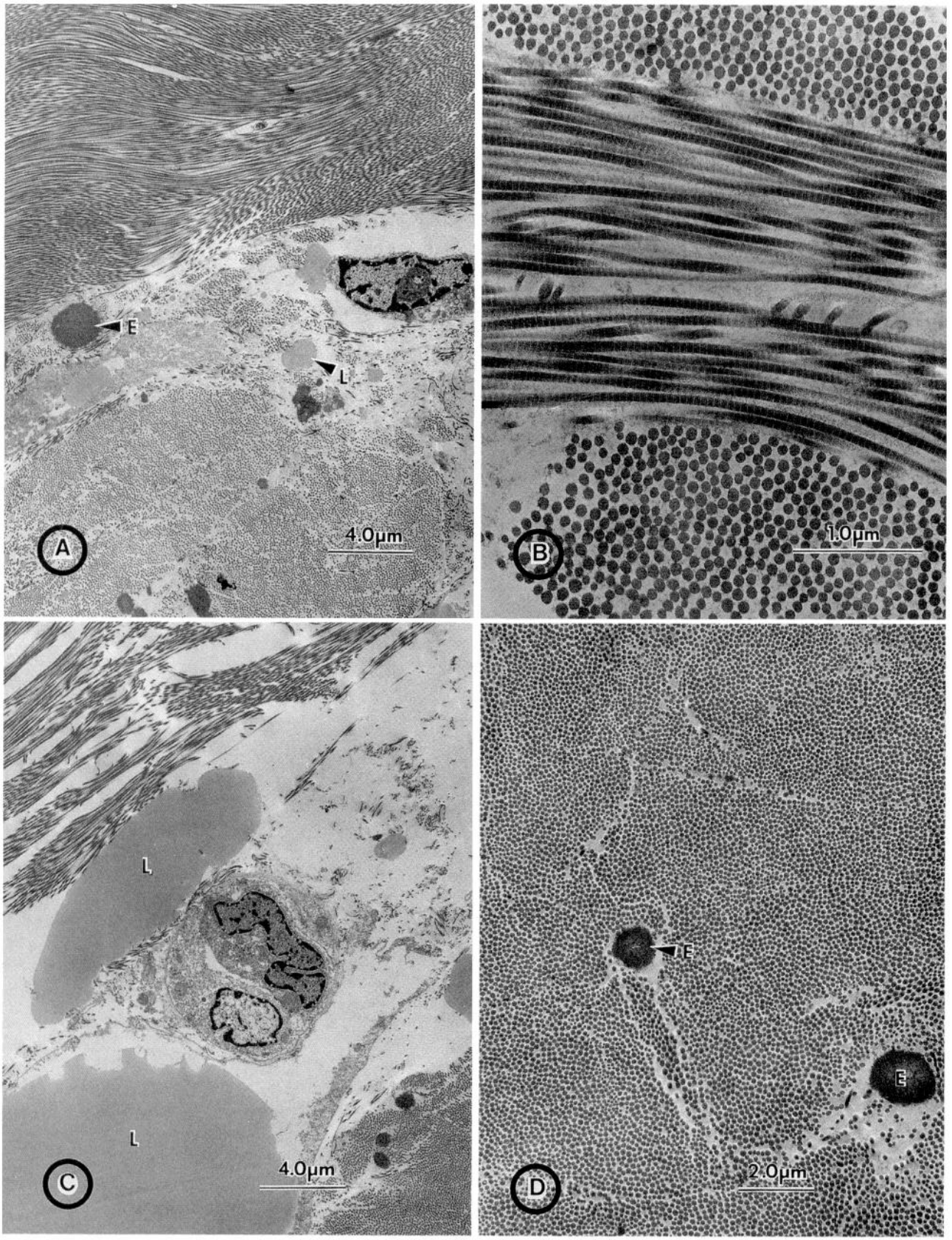

Figure 6. Electron micrographs of blubber from *Megaptera novaeangelae.* (Micrograph A—taken near the epidermis) Large bundles of perpendicularly oriented collagen fibers are interspersed with cells (most of which appear to be fibroblasts), lipid deposits (L), and occasional elastin fibers (E). (Micrograph B) Collagen

mantle. Instead, the bulk of the mantle is composed of muscle. Small amounts of collagen are located in thin sheets, one between the epithelial cell layer of the epidermis and the outer surface of the mantle, the other covering the inner surface of the mantle. In addition, collagen also occurs in an internal network of small bundles of parallel fibers running between the muscle bundles (Fig. 4). The banding periodicity along the octopus collagen fibers was 46.6 nm (±8.6 SD, n = 90), 15% smaller than the periodicity of the St. Augustine collagen.

The muscle fibers that make up the bulk of the mantle are arranged in several layers (Fig. 4). Immediately under a thin external collagen layer is a layer of parallel muscle fibers that runs longitudinally from the mantle edge. These fibers are separated laterally from each other by thin downward extensions of the covering collagen sheet (Fig. 4B). A similar muscle layer, although consisting of larger diameter fibers, is located immediately inside the collagen layer that forms the inner surface of the mantle (Fig. 4D). The center of the mantle is occupied by a space that contains blood vessels (Fig. 4A). Ventrally—between the blood vessel space and the inner, longitudinal muscle layer—lie several layers of muscle fibers that run at a variety of angles oblique to the cross-section (Fig. 4C). Dorsally, between the blood vessel space and the outer longitudinal muscle layer, the muscle fibers generally run in the same direction as the fibers of the outer muscle bundles (Fig. 4B). Lastly, the outer and inner collagen sheets are occasionally connected to each other by thin, radial muscle bundles that traverse the width of the mantle, perpendicular to the rest of the musculature and attached to the collagen sheets by a junctional complex (Figs. 4A, B, C; 5D). All of this structure is supported by occasional thin bundles of parallel-running collagen fibers (Fig. 5A, B). Altogether, there is nothing in the octopus mantle morphology that resembles anything in the two relics.

The mantle muscle cells are basically hollow tubes, tapered at each end. The contractile proteins are arranged in bundles that are rectangular in cross-section and radiate out from the hollow center of the cell like tightly packed spokes (Fig. 5A). The nucleus and mitochondria are located in the center of the cell. The arrangement of sarcomeres in the octopus mantle muscle gives the appearance of oblique striations in occasional sections, but the contractile filaments within each sarcomere are not striated (Fig. 5C). Thick filaments, reminiscent of paramyosin, surrounded by thin filaments are evident in cross sections of the muscle cells. Squid mantle muscle cells have a similar fine structure (Ward and Wainwright, 1972).

Microscopic examination of the blubber from the humpback whale revealed a massive matrix of collagen fibers present throughout the entire thickness of the tissue (Fig. 6). Fat deposits and poorly fixed cellular structure were evident between layers of collagen fibers in the sections of the blubber taken both close to the epidermis and from the medial region (Fig. 6A, C). Neither fat nor any remnant of cellular structure was found in either the Florida or Bermuda carcass. The sections taken from the inner aspect of the blubber layer contained only collagen fibers in very large bundles, interspersed with occasional larger fibers that appeared to be elastin (Fig. 6D). At all levels of section, the collagen fiber arrangement of the blubber was exactly that of the Florida and Bermuda carcasses, namely, bundles arranged in layers running perpendicular to adjacent layers (Fig. 6A, B, C). The banding periodicity along the whale collagen fibers was 54.6 nm (±5.1 SD, n = 83), essentially identical to the banding of the St. Augustine collagen.

The amino acid analyses of the tissues from the two carcasses were also suggestive of collagen. Glycine accounts for about one third of the amino acid residues in both tissues, and both hydroxylysine and hydroxyproline were present as well; these features are virtually diagnostic of collagen (Table I). However, the amino acid compositions of the hydrolysates of the two carcasses are quite different from each other. In particular, the St. Augustine carcass is very rich in proline (169 residues/1000) and quite low in lysine (0.4 residues/1000), in comparison to both the Bermuda collagen (88 residues/1000 and 10 residues/1000, respectively) and skin collagens from several other species (Table I). Of course, the unusually low lysine values in the St. Augustine sample may be an artifact of a century in formalin. Only whale skin collagen (species not reported in Eastoe, 1955) has proline residues/1000 that are anywhere near those from the St. Augustine collagen (Table I). In addition, the amino acid composition of the St. Augustine collagen is very different from that

fibers arranged in bundles running perpendicular to each other are everywhere throughout the entire thickness of the blubber. The size of the bundles varies, depending upon location within the width of the blubber. (Micrograph C—from the middle of the blubber layer). The lipid deposits (L) in this region of the blubber were larger and more frequent than the other areas examined. The perpendicular arrangement of the collagen bundles surrounding the fat deposits was still evident (lower right hand corner and upper left hand corner). (Micrograph D—taken from the inner aspect of the blubber layer) The collagen bundles were very large in this region. The expanse of collagen fibers shown here are all in cross section, and were bounded by equally large expanses of perpendicularly running collagen fibers. Very few cells or lipid deposits were encountered in this region of the blubber, although elastin fibers (E) were quite common.

Table I

Comparative amino acid compositions of skin collagens of several species and the Bermuda and St. Augustine carcasses (values are amino acid residues/1000 residues)

Amino acid	Bermuda carcass	St. Augustine carcass	Octopus mantle[1]	Squid mantle[2]	Carp[3]	Whale skin[4]	Shark skin[5]
Asp	52	50	53	58	48	46	43
Thr	27	28	28	26	25	24	23
Ser	47	45	52	47	43	41	61
OH-Pro	79	54	95	89	82	89	60
Pro	88	169	101	96	117	128	106
Glu	83	82	64	86	69	70	68
Gly	339	330	324	308	326	326	338
Ala	113	106	100	89	119	111	106
Val	25	18	19	21	18	21	25
Cys	0	0	8	4	0	0	0
Met	0	0	6	8	14	5	18
Ileu	14	11	22	21	11	11	15
Leu	32	28	30	32	22	25	25
Tyr	0	0	5	5	3	4	3
Phe	16	14	8	12	14	13	13
OH-Lys	13.1	15.3	15.7	16.1	7.1	6	5.5
Lys	10	0.4	11	15	25	26	27
His	6	4	3	7	5	6	13
Arg	55	48	58	59	52	50	51

[1] Pepsin-extracted collagen from *Octopus vulgaris* body wall (Kimura *et al.*, 1969).
[2] Pepsin-extracted collagen from *Todarodes pacificus* body wall (Kimura *et al.*, 1969).
[3] Gelatin from skin (Piez and Gross, 1960).
[4] Whale skin gelatin, species not reported (Eastoe, 1955).
[5] 0.5 *M* acetic-acid-extracted skin collagen from *Squalus acanthus* (Piez *et al.*, 1963).

of both squid (*Todarodes pacificus*) and octopus (*Octopus vulgaris*) mantle (Kimura *et al.*, 1969), especially with respect to the proline, hydroxyproline and lysine residues (Table I).

Finally, the ratios of hydrophobic, hydroxylated, and polar amino acids, calculated according to the procedures described by Matsumura (1972), were 183, 353, and 464 for the Bermuda sample and 172, 345, and 483 for the St. Augustine sample. These values mapped both collagens into Matsumura's "S range" along with most striated, structural collagens from both invertebrate and vertebrate species. Interestingly, within the S range, the values for the St. Augustine sample were quite close to those for a frog (*Rana temporaria*) skin, while the Bermuda sample mapped near a number of bovine collagens, as well as chick bone, carp skin, and wallaby tail tendon collagens. The results of this comparison must be viewed with some caution, because the lengthy formaldehyde fixation of the Bermuda and St. Augustine fibers may have caused some changes in the amino acid composition of the protein. In addition, the small amount of tissue we had to work with limited the number of analyses we could do for statistical purposes. Nevertheless, neither of the relic specimens mapped close to invertebrate collagens within the S range, including those of octopus and squid (Matsumura, 1972).

Discussion

The microscopy and amino acid analyses clearly demonstrate that both the St. Augustine and Bermuda carcasses were large pieces (extremely large, in the case of the former) of almost pure collagen. Apparently, both pieces of tissue had been in the ocean long enough, *post mortem*, that bacterial action had recycled all but the most resistant of the proteins. Neither carcass is from a giant octopus nor any other invertebrate, but they are also not from the same species.

The electron micrographs of both the St. Augustine and Bermuda samples show fibers with the characteristic banding pattern of collagen. The width of the repeating unit along the fibers is essentially identical to that of rat tail tendon collagen, and the intraperiod banding, although somewhat indistinct, is typical of collagen. In addition, the whale blubber collagen banding periodicity was the same as that of the St. Augustine collagen, while that of the octopus mantle collagen was much less than any of the other samples. This comparison must be viewed cautiously, however, since the differences in the original fixations may have produced artifacts in the banding patterns.

The organization of the collagen fiber bundles in the two relic samples is typical of dermis from a number of

vertebrate groups, including fish, amphibians, and reptiles, where the bundles are distinctly layered (Moss, 1972). A similar layering pattern of the collagen fibers was nowhere to be found in the octopus mantle tissue we examined here. Instead, the octopus mantle is composed mainly of a complex network of muscle fibers containing only small amounts of widely dispersed collagen fibers, as might be expected of an animal so capable of shape-changing. We found absolutely nothing in the octopus mantle morphology that was comparable to the collagen fiber arrangement in the two carcasses, nor has anything similar been reported in squid or cuttlefish mantle (reviewed by Packard, 1988). In contrast, the similarity between the layering pattern of the collagen fiber support matrix of the humpback whale blubber and the fiber pattern in the carcasses is quite obvious. In addition, unlike the octopus mantle, but very much like the Florida and Bermuda tissues, collagen fibers are the main component of the blubber. The whale tissue we examined here also contained fat deposits and cellular structures that were not present in either the Florida or the Bermuda carcass. However, the humpback whale that provided our blubber sample had only recently expired and did not approach the advanced state of decay of the tissues of the two relics. Thus, the fine structure indicates that both carcasses were actually only the collagenous remains of skin, rather than an entire animal, and the organization of the skins is reminiscent of both lower vertebrates and whale blubber. In addition, the thickness of the St. Augustine carcass [3.5 inches (Webb's letter to Verrill dated Jan. 14, 1897) to 10.5 inches (Webb's letter to Dall dated Feb. 12, 1897)] is consistent with whale blubber.

Collagen fiber diameters within an organism range widely from the very thin fibers typical of cartilage and cornea to the comparatively thick fibers of dermis and tendon. The thickness of the fibers within both our samples is also consistent with the diagnosis of their origin in skin. Furthermore, while the diameter of skin collagen fibers can vary both with the age of the organism and the distance from the epithelium (Flint *et al.*, 1984), the unimodal distribution of fiber diameter and the tight packaging of the fibers within both our specimens are typical of a "non-active skin" characteristic of mammals (including pygmy sperm whale blubber, Craig *et al.*, 1987) and birds. In contrast, collagen fiber diameters from the "active" skins of fish, sharks, and reptiles usually have either a bimodal distribution or a distribution skewed towards larger diameters (Craig *et al.*, 1987).

The amino acid compositions of the hydrolysates of the St. Augustine and Bermuda specimens confirm the collagen identification indicated by our microscopy. In addition, they provide some indication of the phyletic source of the two carcasses. One third of the amino acid residues in both samples are glycine. That large an amount of glycine, taken together with the presence of hydroxyproline and hydroxylysine, is diagnostic of collagen. The absence of methionine from both samples and the extremely low lysine values in the St. Augustine sample are unusual and are, perhaps, the result of exposure to whatever preservation chemicals were actually used. The number of proline residues in the St. Augustine collagen is surprisingly high, and the level of that imino acid may be the most important clue to its specific origin. The denaturation temperature of collagen is directly proportional to its total imino acid content. In particular, the imino acids provide the collagen molecule with a degree of thermal stability required at the elevated body temperatures of the homiothermic species (Rigby, 1968; Hochachka and Somero, 1984). Thus, the collagens from invertebrates and poikilothermic vertebrates are relatively low in total imino acid residues (generally less than 200 residues/1000), while homiothermic vertebrate collagens have a combined imino acid total that is usually higher (210 residues/1000 or greater; for examples see the data tabulated in Kimura *et al.*, 1969; Eastoe, 1955). Thus, the total imino acid content of the Bermuda sample (167 residues/1000), together with the rest of the amino acid composition and morphological data presented above, suggests that the source of that collagen was the skin of a poikilothermic vertebrate. Indeed, the relatively small mass of the carcass is easily within the size range of either a large teleost or an elasmobranch. On the other hand, the elevated imino acid content of the St. Augustine collagen (223 residues/1000) together with its amino acid composition, fine structure, and size of the carcass all indicate that it was the remains of the skin of an enormous warm-blooded vertebrate.

Altogether, and with profound sadness at ruining a favorite legend, we find no basis for the existence of *Octopus giganteus*. We concur with Verrill's (1897e) and Lucas' (1897) final words on the matter, that the St. Augustine sea monster was "the remains of a whale, likely the entire skin [blubber layer] . . . nothing more or less."

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